

**Amendments to the Specification:**

Please replace paragraph [0031] on page 12 with the following amended paragraph:

[0031] Figure 9 describes transcription and translation assays :Fig. **9A 9a**) is a schematic diagram of the transcription and translation assay. SP6 RNAP binds to the SP6 promoter (PSP6) on R023 (T7 RNAP driven by SP6 and T7 promoters) (1) transcribing T7 RNAP mRNA, which is (2) translated into T7 RNAP protein. The T7 RNAP protein then binds the T7 promoter (PT7) on R023 (3) resulting in more T7 RNAP protein (2) and initiating the autocatalytic cycle and an exponential increase in T7 RNAP production. T7 RNAP also transcribes luciferase mRNA from PT7-Luc (4), resulting in an increase in luciferase expression proportional to the amount of T7 RNAP present. In the control reaction (below), the lack of PT7 in R037 (T7 RNAP gene driven by only SP6 promoter) prevents any autocatalytic production of T7 RNAP (3). Fig. **9B 9b**) illustrates data from an *in vitro* coupled transcription and translation (Promega) assay. 250 ng of PT7-Luc was combined with 250 ng of either R023 or R037 in a total reaction volume of 15  $\mu$ l and 0.5 U of SP6 RNAP (Promega) was added and incubated at 30° C. 2  $\mu$ l aliquots were removed at time points indicated and subjected to luciferase analysis as described in Materials and Methods. After an initial lag phase, the R023 reaction resulted in an exponential increase in luciferase expression, verifying the autocatalytic nature of the system.

Please replace paragraph [0169] on page 53 with the following amended paragraph:

[0169] *Luciferase and BCA Assays:* Cells were washed twice with 1 mL PBS followed by the addition of 0.2 mL lysis buffer (PBS with 0.1 % Triton® X-100) before being stored at -70°C. Cells were thawed and 5-20  $\mu$ l of sample were assayed in duplicate on a 96-well plate. Samples were assayed using a Berthold Centro LB960 Microplate Luminometer and Luciferase Assay System (Promega). Standard luciferase assays were performed and transfection data is reported as mass quantities of luciferase protein using a standard curve obtained from serial 10-fold dilutions of a 20 mg/mL Photinus pyralis luciferase standard (Promega). Cell-free luciferase

assays are reported in RLUs. Total protein was quantified using a Pierce BCA assay kit as per manufacturer's instructions.

Please replace paragraph [0170] on page 53 with the following amended paragraph:

[0170] *Immunofluorescence Assays*: BHK cells were plated on glass coverslips in 6-well plates (150,000 cells per well) and transfected with 1.5 µg of plasmid DNA. 24 h post-transfection, cells were washed once with 2 mL PBS-IF (10 mM sodium phosphate, 140 mM sodium chloride, pH 7.4) prior to fixation for 10 min with 2 mL 2% paraformaldehyde. Cells were subjected to three 30 s washes before permeabilization with 0.25% Triton® X-100 in PBS-IF for 5 min. After washing three times for 30 s with PBS-IF, cells were incubated with blocking buffer (10% BSA in PBS-IF) for 1 h, shaking gently at room temperature. Cells were washed three times for 10 min with PBS-IF followed by addition of primary antibody solution comprising a 1:1000 dilution of goat anti-T7 RNAP antibody (a gift from Dr. Paul Fisher at the Department of Pharmacological Sciences, State University of New York at Stony Brook) or 1:1000 dilution of mouse anti-luciferase monoclonal antibody (Abcam) in 2% BSA in PBS-IF. Cells were incubated with primary antibody solution for 2 h while shaking at room temperature. Cells were washed three times for 10 min in PBS-IF followed by the addition of secondary antibody (Rabbit anti-goat IgG, FITC labeled (QED Bioscience Inc) or Rabbit anti-mouse Texas Red labeled (Abcam), 1:200 dilution in 2% BSA-PBS-IF) and incubation for 2 h while shaking at room temperature. Cells were washed four times for 10 min with PBS-IF before being mounted and photographed on a Zeiss Axiovert S100 fluorescence microscope. Percentage of cells transfected was determined by counting transfected and non-transfected cells under the microscope. Data indicate the average of six separate counts from three different experiments.

Please replace paragraph [0172] on page 54 with the following amended paragraph:

[0172] BHK cells were plated on 6-well plates (150,000 cells per well) and transfected with 1.5 µg of R011 or L053 in triplicate. After 24 h cells were treated with 20 µg/mL Actinomycin D. At 0, 2, 4, 6 or 8 h after Actinomycin D treatments, cells were washed once with PBS and

recovered by trypsinization. Cells from triplicate wells were pooled before harvesting total RNA (RNeasy miniprep kit, Qiagen). 10, 5 or 2.5 µg of total RNA was subjected to RNase protection analysis using the RiboQuant™ RPA system (Pharmingen) according to the manufacturers protocol. All values shown are the average +/- standard deviation of two independent experiments. Data was collected using a Typhoon Phosphoimager (Amersham Biosciences) and analysis was performed using ImageQuant software (Amersham Biosciences).